Characterization and identification of bacterial sRNAs and their involvement in virulence regulation

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Introduction

- The discovery and characterization of **RNA regulators** (sRNAs) in bacteria has emerged as one of the main post-transcriptional processes (1).
- They belong to two classes: **cis-encoded sRNAs** and **trans-encoded sRNAs.**
- Some trans-encoded sRNAs require a **chaperon** called **Hfq** to perform the hybridization of the sRNA with the mRNA target (2).
- The relevant importance of sRNAs in virulence regulation is only described in *Escherichia coli* and other species related. Since there has been a reduction in the **cost of transcriptomics** more experiments have been reported in other pathogen species to identify novel sRNAs involved in virulence regulation.

Aims

- Describe different strategies used to identify and characterize novel sRNAs.
- Identify the targets of novel sRNAs using computational and experimental methods.
- Characterize different approaches to assign a particular role of a sRNA.
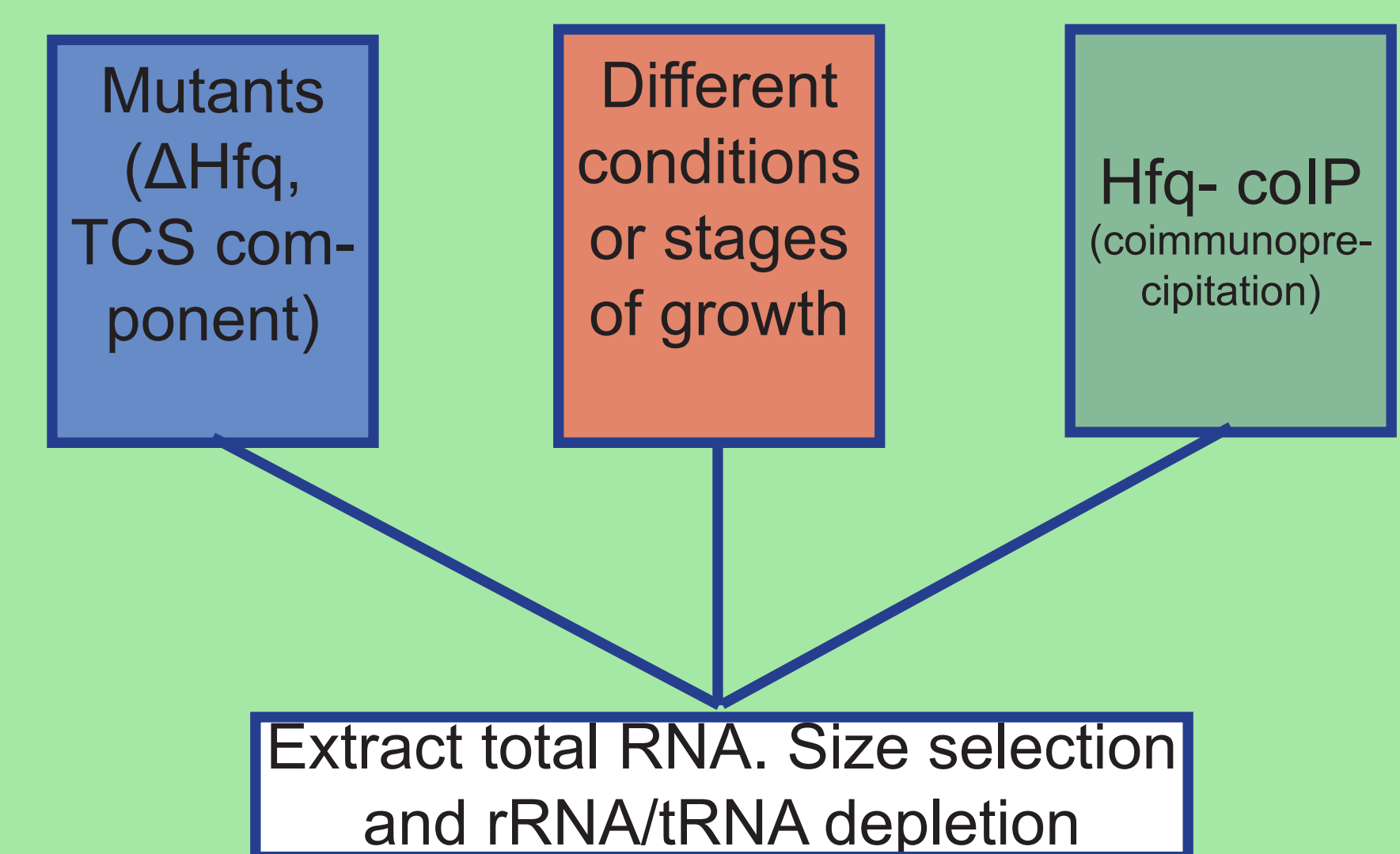
Characterization and identification of sRNAs

Mutants (ΔHfq, TCS component)

Different conditions or stages of growth

Hfq- coIP (coimmunoprecipitation)

Extract total RNA. Size selection and rRNA/tRNA depletion

Table 1. Comparison between transcriptomic methods (3)

|	Tiling arrays	cDNA seq	RNA-seq
Principle	Hybridization	Sanger sequencing	High-throughput sequencing
Resolution	From several to hundred pb	Single base	Single base
Dynamic range to quantify gene expression	Up-to a few hundred-fold	Not practical	>8000 fold

Filtering data (4)

- Run a BLAST **looking for homologs**
- Eliminate all the sequences within known ORF
- Focus on **intergenic regions** or running antisense in known ORF
- **rho-independent** transcription terminator

Confirmation of sRNA by Northern blot and RT-PCR

Target validation

Target RNA2/ TargetRNA/ INTARNA (5)

- Regions more conservated in sRNA
- Regions with more accesibility in sRNA or candidate mRNA target
- Energy of hybridization
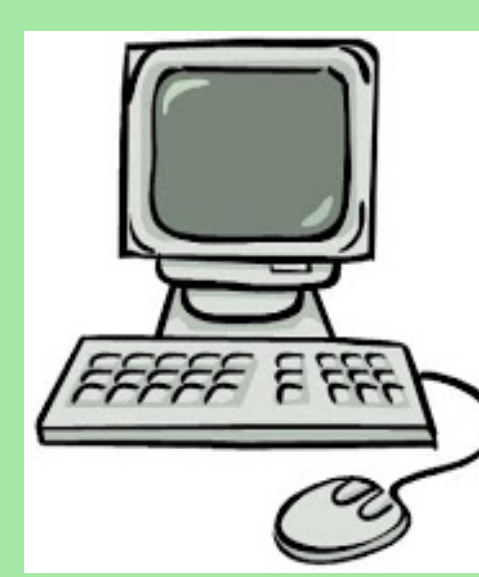
Candidate mRNA targets

Pulse overexpression of sRNAs (6)
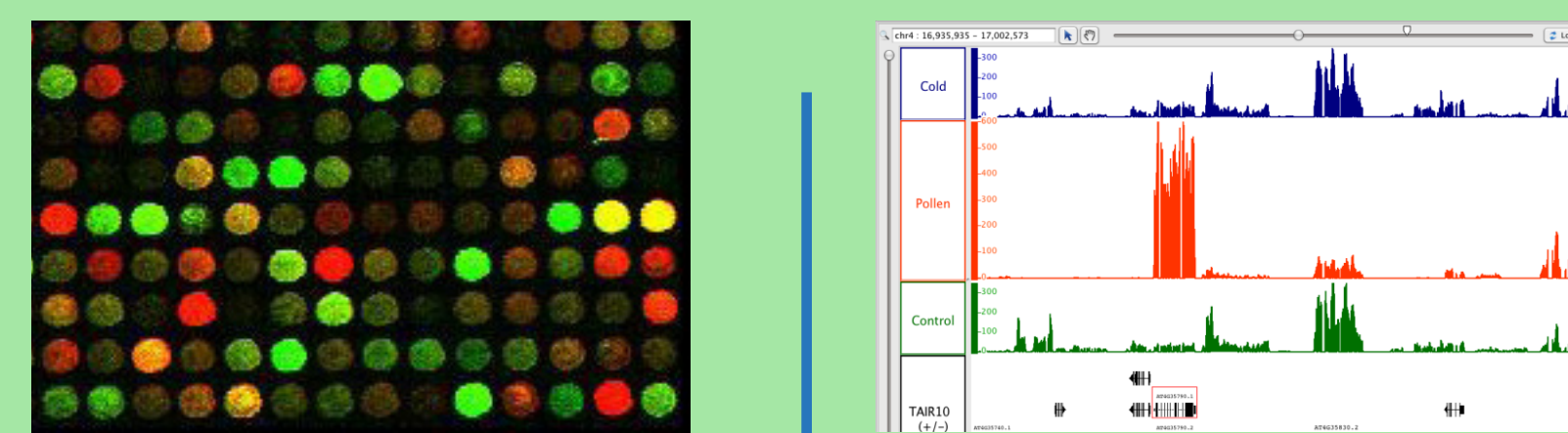
Plasmid with inducible promoter

Plasmid with inducible promoter + sRNA

Grow to log or early stationary phase

Induce sRNA expression for 10 min

sRNA recognizes target and induces changes in expression

Compare mRNA profile expression

GFP reporter system to confirm mRNA target

Virulence implication

During an infection, **monitoring the levels of sRNA** could reveal the patterns of regulation followed by a microorganism (Figure 1). In different stages of infection it is necessary that some genes quickly increase their expression level, thus considering regulation mediated by sRNAs is a good methodology to detect sRNAs involved in virulence (7).
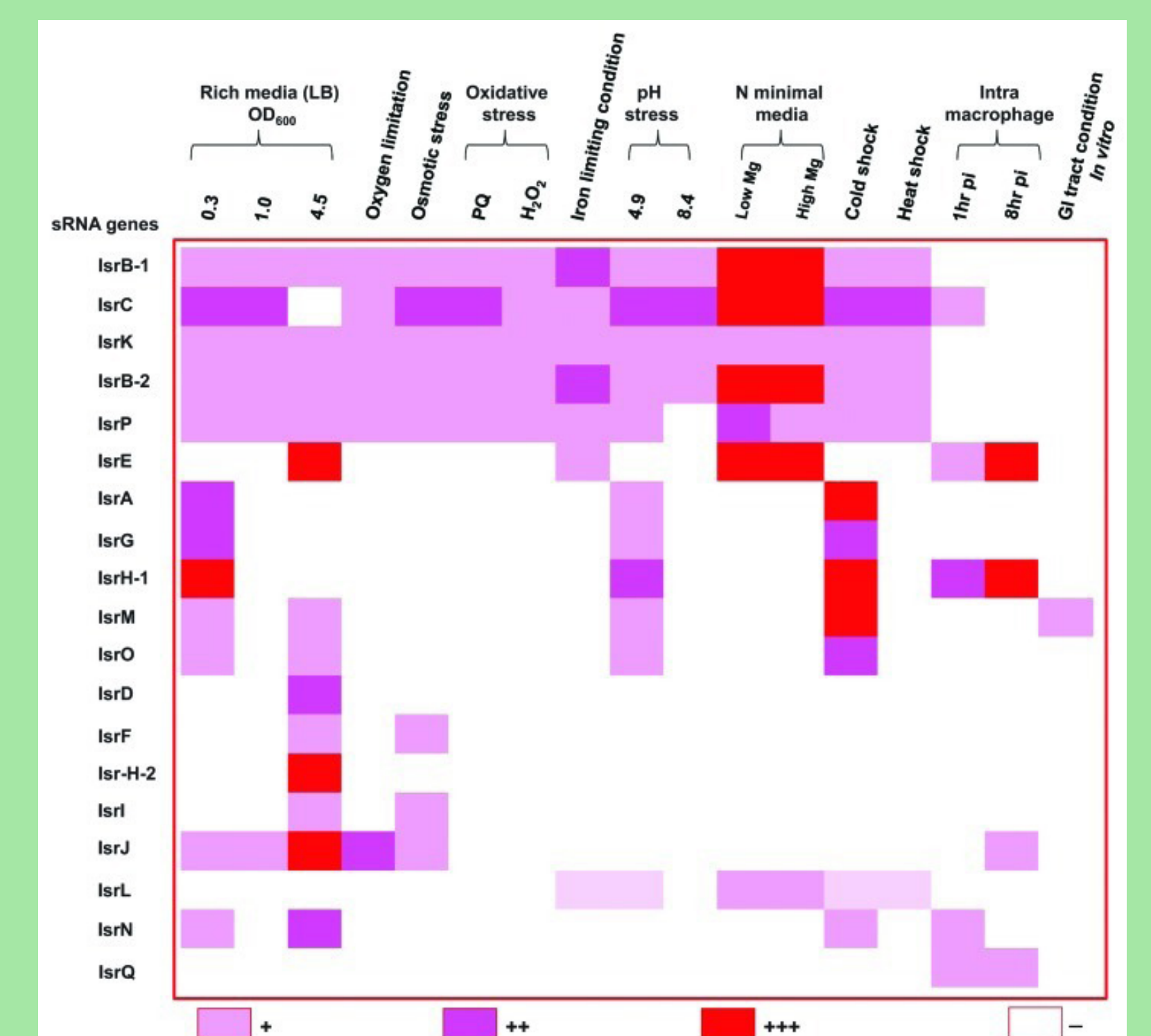

Figure 1. Monitorization of RNA levels in different simulated conditions and media using Northern-blot to quantify RNA expression (7)

Selection of strong sRNA candidates are considered to construct **knock-out mutants** by the red-recombinase method or homologous recombination. **Targeted sRNA deletions** are tested to measure the relative fitness of mutants using different approaches such as **Tn-seq, survival rate, lesion diameter** (Figure 2) or **measure of weight** during the experiment (8).
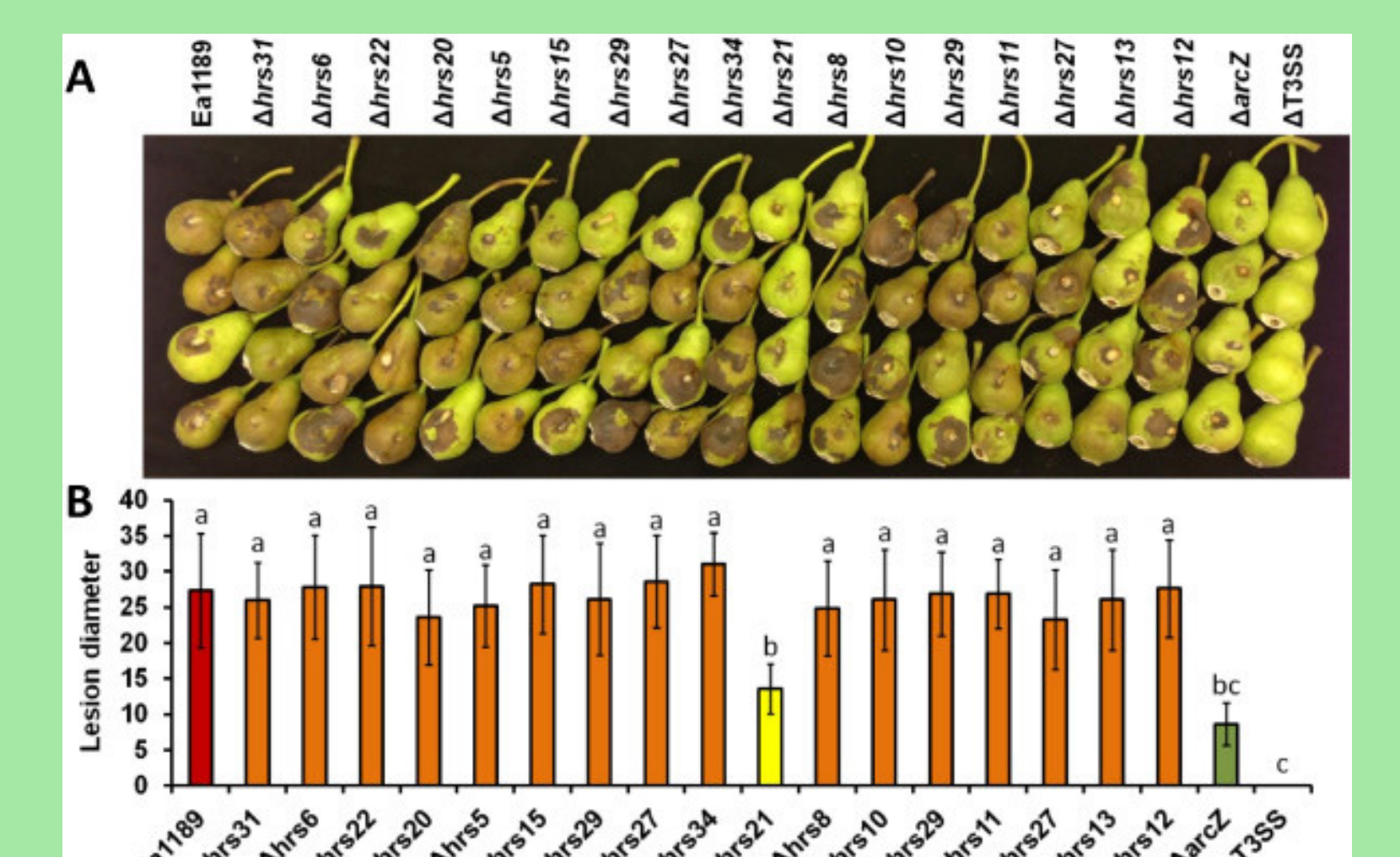

Figure 2. Lesion diameter using wild-type and mutants of *Erwinia amylovora* in immature pear fruits (8)

Conclusions

- **RNA-seq** has become the most useful technique to identify novel sRNAs. It **detects and quantifies** the level of sRNA in complex environments where it is difficult to obtain high levels of RNA.
- During the next years, the pools of sRNAs available in bioinformatics databases as well as novel sRNAs identified through homology to related species will increase exponentially.
- **TargetRNA2** is the most useful computational tool to **integrate the data from RNA-seq** and to reduce false positive rates.
- **Pulse-overexpression of sRNA** is the main approach used to **confirm the pool of targets** identified through computational methods.
- Generating different **knock-out mutants** can confirm an attenuation of **virulence** using different animal or plant models. Even though, it is complicated to observe a clear phenotype if there are other sRNAs involved in the regulation of the same gene.

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